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Antibodies
Volume I
a practical approach

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Preface

Under the title *Antibodies — A* of two, IRL Press is extending in the biosciences into the sphere binding properties of antibodie the last 20 years have seen an e laboratories occupied in the stud and biochemical level that hav preferred and often essential t biological properties of antigen of applications is enormous — differentiation markers, serum associated antigens, microbial fective across such a diverse r two closely linked areas of ex reagents that perform reliably assay procedures appropriate t aspects of antibody methodolog with how to use them. I have of several of my colleagues in th necessary have included contribu perspective. The result is a pra lasting value to those many monoclonal antibodies, and to t who may need guidance on antib the chapters on antibody metho of most value as bench aids. T that are used routinely and ha agglutination, ELISA and rad peroxidase methods and affinit application but of major impor tissue typing, the technologies porary appraisal of methods; a activated cell sorting and the us importance and clearly have g

Preface

Under the title *Antibodies — A Practical Approach*, of which this volume is the first of two, IRL Press is extending its highly successful Practical Approach series of texts in the biosciences into the sphere of immunological methods. The specificity and antigen-binding properties of antibodies have been exploited since the start of the century but the last 20 years have seen an enormous growth in their application. There can be few laboratories occupied in the study of living systems and cell products at the physiological and biochemical level that have not come to appreciate the value of antibodies as the preferred and often essential tools to identify, quantify and probe the structure and biological properties of antigenic molecules in the context of their own work. The range of applications is enormous — animal and plant hormones, enzymes, cell receptors and differentiation markers, serum proteins, tissue and cell-specific antigens, tumour-associated antigens, microbial and parasite antigens and many, many others. To be effective across such a diverse range of applications the use of antibodies relies upon two closely linked areas of expertise — that of producing highly specific antibody reagents that perform reliably, and that of choosing and performing reliable antibody assay procedures appropriate to the task. This book has brought together these two aspects of antibody methodology; linking how to produce and quality control antibodies with how to use them. I have drawn heavily upon the expertise and long experience of several of my colleagues in the Department of Immunology in Birmingham but where necessary have included contributions from experts of other centres to achieve a broader perspective. The result is a practical book in two volumes which will, I hope, be of lasting value to those many scientists who need to produce either polyclonal or monoclonal antibodies, and to the larger number of scientists, students and technicians who may need guidance on antibody methods. As intended in a practical approach series the chapters on antibody methods adopt a bench manual format with the aim of being of most value as bench aids. The methods described are of three categories — those that are used routinely and have wide application, such as reactions in gels, haem-agglutination, ELISA and radioimmunoassays, immunofluorescence and immuno-peroxidase methods and affinity purification of molecules; those of more restricted application but of major importance in the clinical field, such as red cell typing and tissue typing, the technologies of which continue to advance with a need for contemporary appraisal of methods; and recently developed methods, such as fluorescence activated cell sorting and the use of antibodies in tumour imaging, that are of growing importance and clearly have great future potential.

D. Catty

CHAPTER 2

Production and quality control of polyclonal antibodies

DAVID CATTY and CHANDRA RAYKUNDALIA

1. INTRODUCTION

As different tests require different properties of antisera there can be no single procedure for animal immunization that guarantees an ideal product for all requirements. Nevertheless certain principles can be adopted which together form the 'ground rules' for antiserum production; ideal reagents rely to some degree on trial and error as every immunogen is different and every animal responds individually. The essential properties of antisera are high titre coupled with high average avidity and specificity. The former, which are influenced by the use of various adjuvants, depend upon achieving a subtle balance between widespread stimulation of antigen-sensitive cells of the animal's lymphoid tissues, continued presence of immunogen to allow extensive polyclonal proliferation of plasma cells but, within this, a competition between cells for limiting amounts of antigen so that only the highest affinity cells will respond. The latter depends critically upon the purity of immunogen administered, as minor contaminants may induce a disproportionately large antibody response.

Many antisera are produced for their precipitating properties and since this depends upon efficient lattice formation between antibodies and several antigen determinants, both multiple specificity and a balance in the titre of antibodies is important. Antisera that can be used in high dilution are always preferable to reduce background activity. In 'rocket' and two-dimensional immunoelectrophoresis (RIEP and 2D-IEP respectively) it is essential to have antibodies in the agar gel of narrow isoelectric range and the choice of immunized species is important here—sheep IgG_1 antibodies have especially good qualities in this respect.

Where antisera are to be used in more sensitive quantitative assays such as enzyme-linked immunosorbent assay (ELISA) or radioimmunoassay (RIA) there are more stringent requirements for high avidity and specificity. Antiglobulin reagents must bind only to the target species immunoglobulin, or to a particular isotype of that species if required, and scrupulous absorption of species and isotype cross-reactive antibody components will be needed to achieve this.

Most applications of RIA are for measurement of small molecules at low concentration. For immunization, small molecules, having low intrinsic immunogenicity, require coupling to larger protein carriers. Methods for such coupling are important considerations in determining the specificity of the response. Careful selection from several antisera may be needed to find one that has suitable properties.

The harvesting of an antiserum is only the start of obtaining a useful working reagent.

For most assays the only relevant part of the serum is what lies in the immunoglobulin fraction—the rest can be conveniently removed. This increases the ratio of antibody to total protein, reduces background reactions and increases sensitivity of assays. Immunoglobulin separation is also the initial step for fluorochrome, enzyme and radioisotopic labelling of antibody, and in affinity purification.

After initial tests in specificity and titre, quality control steps may involve absorption of unwanted antibodies as an alternative to affinity purification. This is best performed with insolubilized antigen polymers or beads, as fluid absorption forms soluble immune complexes with unfortunate properties.

Once the specificity and titre of the antibody is satisfactory it can be calibrated against a reference reagent and finally tested across a range of immunoassays in which it might be applied. *Table 1* summarizes the strategy for antibody production and quality control.

2. GROUND RULES IN ANTIBODY PRODUCTION

2.1 **The immunogen**

2.1.1 *Small molecules*

Molecules of less than 5 – 10 000 mol. wt require conjugation to a carrier protein. The antibody response will be directed towards the carrier determinants as well so choose a carrier that is irrelevant to future assays and/or that can be prepared easily as an adsorbent. Although use of a protein carrier from the immunized species might seem a useful tactic this is inadvisable as antibody responses to most (thymus-dependent) immunogens require recognition of carrier determinants by helper T cells. Bear in mind that the size, charge and polar or non-polar properties of haptens are important in inducing the antibody responses and these properties can be influenced by the extent of uniformity and density of conjugation of hapten to carrier and by the choice of carrier. It may be necessary to prepare the conjugates with several carriers and with a range of hapten-carrier coupling ratios and to try all these to find the best constructed immunogen. In principle, antibodies to larger haptens may be hapten-specific but these are usually also heterogeneous. The choice of coupling reaction and to which group of the hapten (see Section 3.1), is critical to hapten orientation, and will influence antibody specificity, which is usually away from the coupling site. Antibodies to smaller haptens may incorporate specificity to the link region. It may be necessary to conjugate using a 'linker' group and in any event the anti-hapten specificity should not be tested with the same conjugate but with free hapten [e.g. in RIA or haemagglutinin inhibition (HAI)] or hapten conjugated in the same way to a different carrier.

2.1.2 *Large molecules*

Those with a higher degree of conformation and structural rigidity tend to be stronger immunogens with immunodominant regions and multiple antigenic determinants which induce precipitating antiserum. The use of adjuvant is nevertheless recommended for these molecules to obtain the best results.

Table 1. Strategy for antibo

1.	*Preparation of imm*	
	(a)	Purify.
	(b)	Test purity
	(c)	Conjugate t
2.	*Immunization* (Secti	
	(a)	Select an ap
	(b)	Decide on th dose and tim planning is
	(c)	Prepare the for a single
3.	*Test procedure* (Sec	
	(a)	Test bleed a
	(b)	Check specil for final ap
	(c)	Do trial absc
	(d)	Retest absor
	(e)	Decide on t
	(f)	Repeat (a) t
4.	*Harvesting, storage*	
	(a)	The volume
	(b)	Store in alic
	(c)	Test- and tr
5.	*Immunoglobulin frac*	
	(a)	Prepare by t
	(b)	Test the pur
	(c)	Check the a
	(d)	Conditions
6.	*Affinity purification*	
	(a)	Check the p
	(b)	Prepare the
	(c)	Check antig
	(d)	Perform tria
	(e)	Perform bul
7.	*Quality control* (Sec	
	(a)	Assess the p where avail
	(b)	Calibrate ag
	(c)	Quantitate a
	(d)	Determine i

2.1.3 *Foreignness*

There is an overriding r
animal. Mammalian ser
plex, are poor immuno
adjuvant and T helper c